EXHIBIT D

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Structure and Chemistry of the Apatites and Other Calcium Orthophosphates

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PREFACE

The apatites and related calcium phosphates have been of considerable interest to biologists, mineralogists, and inorganic and industrial chemists for many years. The reasons for this are clear: the apatites form the mineral component of bones and teeth, and the more acid calcium phosphates are probably also involved in mineralisation processes; many of the calcium phosphates occur in pathological calcifications; apatites and other calcium phosphates are increasingly used as biocompatible materials for bone replacement or for the coating of bone prostheses; they are widely distributed minerals, providing the world's supply of phosphorus, particularly phosphates for the production of fertilisers; and they are used as phosphors in fluorescent light tubes. The great versatility of apatites in accepting a large variety of substitutional ions complicates their study, but also presents considerable interest in its own right.

During the last thirty years or so, substantial progress has been made in the understanding of the structure and chemistry of the apatites and related calcium phosphates. Although, the basic apatite structure was known at the beginning of this period (its structure had been determined in 1930), important aspects of the structures of the other calcium orthophosphates were unclear; even the existence of one of them, octacalcium phosphate, had barely been universally accepted and little was known about the amorphous calcium phosphates. As regards the apatites, there were still heated disputes about the location of the carbonate that biological, many mineral and synthetic apatites contain. Another major puzzle was that apatites could be precipitated from solution with Ca/P molar ratios from 1.667 (that for hydroxyapatite) to about 1.5, without any apparent changes in the X-ray diffraction powder pattern.

Single crystal X-ray diffraction studies have provided much of the detail of the structures and crystal chemistry of the apatites and other calcium phosphates. However, progress in understanding the relationship between carbonate and apatites and the variable Ca/P ratios of precipitated apatites has had to rely on indirect methods because of the absence of suitable single crystals. Infrared spectroscopy, which was little used before the beginning of this period, and more recently, nuclear magnetic resonance spectroscopy have probably been the most useful.

Another area of progress has been in studies of the solution chemistry of the calcium phosphates. The aqueous phase diagram in the neutral and alkaline dilute region is now accurately known, as well as some aspects of the processes of their nucleation, crystal growth and dissolution. However, much remains to be understood particularly, how these are affected by interactions with other

(Section 1.8.2) and can then be heated at 900 °C for 2 h with the confident expectation that the reaction will go to completion.

α-TCP can be made from β-TCP by aging above 1200 °C [16] or by heating at 1400 °C, well above the transition temperature, as in the preparation of single crystals (see later).

Powders prepared in solution

β-TCP does not form in aqueous systems under normal laboratory conditions (*i.e.* up to 100 °C and at atmospheric pressures). However, whitlockites can be precipitated in such systems with the following molar % substitution of Ca^{2+} ions in solution: Mg^{2+} 0.1 to 1; Mn^{2+} about 1; and Fe^{2+} about 10 (Al^{3+}, Fe^{3+}, Ni^{2+}, Co^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+} and Ba^{2+} were not effective) [299]. It has been shown from X-ray and ESR work (Section 1.6.2) that Mg^{2+}, Fe^{2+} and Mn^{2+} ions can occupy the Ca(5) sites. By contrast, at least in β-TCP, Cu^{2+} ions occupy Ca(3) sites (Section 1.6.2). The substitution of an ion somewhat smaller than the Ca^{2+} ion into the six-fold coordinated Ca(5) site probably increases the stability of the lattice because Ca^{2+} ions usually have a higher coordination number. The substantially reduced solubility (Section 1.6.5) and raised β to α transition temperature (Section 1.6.6) of β-TCa₃MgP compared with the magnesium-free compound is further evidence of this increased stability. It has been suggested that Mg^{2+} ions must comprise at least ~5 % of the cations in whitlockite for it to be sufficiently stable to precipitate in aqueous systems [248].

Magnesium whitlockites have been prepared by the dropwise addition of 250 ml of a solution containing Ca^{2+} and Mg^{2+} ions (total Ca + Mg = 10 mmol l⁻¹) into 750 ml of a stirred phosphate solution (10 mmol l⁻¹) at various temperatures, followed by a 4 h digestion, filtration and drying [269]. Magnesium whitlockite, uncontaminated by other phases, can be obtained by this method from solutions with a Ca/Mg molar ratio of 0.25 and a starting pH of 5 (RZ LeGeros, personal communication, 1990). The slow simultaneous addition of a mixed CaCl₂ plus MgCl₂ solution and a Na₂HPO₄ solution to a large volume of water at 100 °C for various fixed pH values from 5.5 to 9 (concentrations and volumes not given) has also been used [248]. This latter procedure has the advantage that the conditions of precipitation will be more uniform during the course of the preparation, but both procedures suffer from the formation of apatite, amorphous products and variations in the crystallinity of the whitlockite to an extent that depends on the exact experimental conditions. The most important determinant of the type of CaP obtained is the solution Mg/P molar ratio; a ratio of 0.05 favours the precipitation of whitlockite, and this is better crystallised if formed at 100 °C [269]. Acidic conditions (pH 5 to 6) also favour a better crystallised whitlockite [248,269], a finding that might be

related to the fact that HPO_4^{2-} ions must be present in order to be incorporated into the whitlockite lattice. Whitlockite has also been prepared at 37 °C by the dropwise addition of 500 ml of an alkaline solution of Na₂HPO₄ to 500 ml of an alkaline solution of a mixture of calcium and magnesium acetates in the stoichiometric amounts required to form 10 g of $Ca_{20-n}Mg_nH_2(PO_4)_{14}$, where $1.4<n<3.8$ [300]. The hydrolysis of DCPD [248,269,301] (Section 1.4.6) and DCPA [269] (Section 1.5.5) in the presence of Mg^{2+} ions has also been used for whitlockite preparation. The constant composition method (Section 3.8) has been used to study the crystallisation of whitlockite on whitlockite seeds at 37 °C and pH 6.00 from metastable calcium-magnesium phosphate solutions [302]. The formation of whitlockite was confirmed. Whitlockite has occasionally been found during the hydrothermal formation of bone implants in phosphate solutions from coral skeletal calcite that contained magnesium [303] (Section 4.5.6).

Contractions of the α- and c-axis parameters with increasing magnesium content occur in synthetic whitlockite, but these are less than the contractions observed between β-TCP and β-TCa₃MgP for the same magnesium content [248]. The magnesium content in synthetic whitlockite is generally insufficient to take the c-axis parameter contraction beyond the point when, by analogy with β-TCa₃MgP (Section 1.6.2), it would start to expand.

The maximum reported magnesium content incorporated into the lattice for a synthetic whitlockite preparation is about 12 % of the total cations [248]. This compound was made by the simultaneous addition of a mixed (Ca,Mg)Cl₂ solution with a Ca/Mg molar ratio of 5 and a Na₂HPO₄ solution to a large volume of water at 100 °C and pH 5.5 to 6 as described above. Thus it seems possible to fill all the Ca(5), and probably some of the Ca(4) sites, by Mg^{2+} ions if appropriate conditions are used (the Ca(5) sites comprise 10 % of the total cation sites in the Mg whitlockite end-member, Formula 5, Table 1.5). Interestingly, it was also reported [248] that the axial parameters of some samples of whitlockite in pathological calcifications from tracheal and disc cartilage indicated an even greater magnesium incorporation.

Single crystals

Single crystals of α-TCP have been prepared by heating stoichiometric amounts of finely ground DCPA and CaCO₃ with 1 wt % cornstarch and a few drops of water at 1400 °C for two days (*i.e.* above the β-TCP to α-TCP transition at 1125 °C, Section 1.6) [283]. For the preparation of single crystals of β-TCP, α-TCP (prepared as above) was heated below the transition for seven days at 1100±10 °C [286]. These crystals were used in the structure determinations of α- and β-TCP discussed in Section 1.6.2.

Single crystals of β-TCP and two β-TCa₃MgPs with different magnesium contents have been made by grinding alcoholic slurries of CaCO₃ (0.58, 1.79

solid state reactions at high temperatures, usually between equimolar quantities of DCPA and $CaCO_3$, (Reaction 1.14). This reaction should be carried out in a dry atmosphere or vacuum, or with rapid cooling (to prevent uptake of water and formation of OHAp, Reaction 1.15). The borderline at which the reverse of Reaction 1.15 occurs is at 1575±10 and 1648±10 K for water vapour partial pressures of 73.6 and 760 mm Hg (9.81 and 101.3 kPa), respectively [19,21].

$$2CaHPO_4 + 2CaCO_3 \rightarrow Ca_4(PO_4)_2O + 2CO_2 + H_2O \qquad 1.14$$

$$3Ca_4(PO_4)_2O + H_2O \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 2CaO \qquad 1.15$$
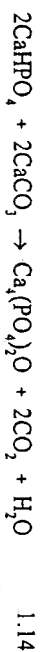
In one study, pure (from an XRD point of view) TetCP was synthesised by heating homogenised equimolar quantities of DCPA and $CaCO_3$, in air, or in a flow of purified nitrogen [319]. Those samples heated in air were subsequently cooled over silica gel; the others were cooled in nitrogen. Pure TetCP could be synthesised in air by heating for 6 h at 1300 °C, followed by 4 h at 1400 °C. Uncontaminated TetCP was also formed if the mixture was heated in air at 1200 °C for 2.5 h, followed by 23 h at 1300 °C in nitrogen. In another preparation, stoichiometric amounts of $Ca_2P_2O_7$ and $CaCO_3$, were heated at 1300 °C for 6 h, and then quenched to room temperature [284,318].

The single crystal (~0.33 × ~0.11 mm²) of TetCP that was used for the structure determination described above was selected from a sample prepared by heating an equimolar mixture of DCPA and $CaCO_3$, in a platinum foil envelope at 1500 °C for 24 h in vacuum [312].

Optical properties and habit

TetCP has optical constants (sodium light) as follows: biaxial (positive), α = 1.643, β = 1.645 and γ = 1.650 and calculated 2V of 66 ° [16]. It occurs as microscopically small pseudo-orthorhombic prisms of simple form, but is frequently granular and anhedral; polysynthetic twinning on (010) and (001) is common [16]. This twinning is described in more detail in ref. [312].

Other properties

TetCP reacts readily with water vapour at temperatures between 1100 and 1420 °C (Equation 1.15) [272,284,321-323]. Reaction temperatures at two water vapour pressures were given above. The reaction has been studied by IR, TGA and XRD [284,318]. TetCP melts at 1720 °C [273] (Fig. 1.10).

TetCP is more basic than OHAp and has a higher catalytic activity and selectivity for alcohol decomposition [284]. It was found to be stable in water at room temperature for four weeks, but at 80 °C, hydrolysis to OHAp was

1.8 Amorphous calcium phosphates
1.8.1 Introduction

Amorphous calcium phosphate (ACP) shares with OCP the fact that there were early reports of its occurrence in the literature [9] but it was not until the mid 1960's that its formation and structure were investigated in detail. ACP is of interest because it often occurs as a transient phase during the formation of CaPs in aqueous systems, particularly ns-OHAps (Section 1.8.4). It was also reported [325] that bone contains ACP as a separate phase whose concentration is higher in younger animals than older ones, but later investigations indicated that ACP is not present in bone [326] (Sections 4.6.1 and 4.6.2). However ACa_3CO_3Ps have been found in the calcareous corpuscles of tapeworms, in the tips of lobster claws and in various pathological tissues [268]. Some of the literature on the structure and properties of ACP up to 1974 (a period in which most of the basic chemical work was done) has been reviewed [327]. Detailed studies have been made of the thermal decomposition and solid state conversion of ACP into apatitic salts [92,328,329], ACa,MgP [92,328] and ACP containing $P_2O_7^{4-}$, CO_3^{2-} or F^- ions [92]. Other papers and more recent work up to 1986 have been cited in ref. [330]. The formation of a second kind of ACP (ACP2), in addition to the usual form (ACP1), has been proposed to explain the variation of pH during the transformation of ACP into crystalline CaP phases [331] (Section 1.8.4). The synthesis of an amorphous OCP has been reported [146] (Section 1.8.3).

1.8.2 Preparation

ACP has been prepared at 25 °C by the rapid addition, with stirring, of a solution of $(NH_4)_2HPO_4$ (0.25 mol l⁻¹) to a solution of $Ca(NO_3)_2.4H_2O$ (0.75 mol l⁻¹) to achieve a final phosphate concentration of 0.15 mol l⁻¹ and a Ca/P molar ratio of 1.71 [332]. All solutions were adjusted to pH 10 with concentrated NH_4OH prior to mixing, and the reaction carried out in a closed system to reduce CO_2 contamination. The initial solid phase, formed immediately on mixing, was separated and washed with ammoniated water at pH 10, then freeze-dried. The Ca/P molar ratio of the solid was very reproducible at 1.52. Similar preparations, also at high pH, have been reported [92,298,328,329].

ACP has also been prepared under conditions different from those given above, particularly at lower pH values and/or with more dilute solutions, but usually with rapid mixing and separation of the solid phase (generally shortly after initial solid formation), followed by freeze-drying [92,298,326,328,329,333-338]. Rapid precipitation from alkaline alcoholic solutions has also been used [146] (Section 1.8.3).

The induction time before the initial formation of ACP is highly sensitive

$[Ca] \times [P]$ products or Ca/P molar ratios, higher temperatures or pH values, or by a lower dielectric constant or the presence of $P_2O_7^{4-}$, F$^-$, Mg^{2+} or CO_3^{2-} ions or collagen [333]. The time is increased by citrate, poly-L-glutamic acid or polyacrylate [333].

ACPs with CO_3^{2-}, Mg^{2+} ions etc. ACPs can also be prepared with significant and even substantial amounts of other ions in addition to Ca^{2+} and phosphate ions. Formation of ACP by the methods discussed above in the presence of large amounts of carbonate leads to its incorporation in the solid in amounts that increase with increasing pH [339] and initial solution CO_3/P ratio [92,339]. The addition of Mg^{2+} or citrate ions to the precipitating system enables ACP to be prepared under more acid conditions than would otherwise be possible [340,341]. Thus an ACP can be prepared [341] at pH 6 and ambient temperature by adding a phosphate solution (KH_2PO_4, 48, K_2HPO_4 22 and KNO_3, 81 mmol l^{-1}) to an equal volume of a calcium solution ($Ca(NO_3)_2$ 32, KNO_3, 81 and potassium dihydrogen citrate 6 mmol l^{-1}). The pH was then raised to 6, and maintained there during precipitation by the addition of KOH solution. The ACP was separated and freeze-dried. It was suggested that ACP could form under these more acid conditions because the added ions inhibited the growth of crystalline phases [341]. In studies of $ACa,MgPs$ prepared at pH 11.2 and with a (Ca + Mg)/P molar ratio of 1.5, no segregation of Mg^{2+} ions between solid and solution was found for a wide magnesium concentration range [92]. ACP has also been synthesised at 37 and 100 °C by the dropwise addition, with stirring, of 250 ml of a calcium acetate solution (0.02 mol l^{-1}) to 750 ml of a phosphate solution (0.016 mol l^{-1}) containing pyrophosphate or CO_3^{2-} ions [268]. In some preparations, Ca^{2+} ions were replaced by Mg^{2+}, Ba^{2+} or iron ions on a mole for mole basis [268]. At 37 °C and pH in the range 7 to 9, the order of efficacy in promoting the formation of ACP was $Mg^{2+} + P_2O_7^{4-} > P_2O_7^{4-} > Mg^{2+} + CO_3^{2-} > CO_3^{2-}$ [268]. In contrast to magnesium, pyrophosphate is preferentially incorporated in the solid phase: the ratio (pyrophosphate)/(pyrophosphate + orthophosphate) is up to 10 % higher in the precipitated ACP compared with this ratio in the solution [92]. The formation of ACa,CO_3P from DCPA [267,268] has already been noted (Section 1.5.5). The presence of Mg^{2+}, pyrophosphate or CO_3^{2-} ions greatly increases the stability of ACP against conversion to apatitic salts (Section 4.5.1). The precipitation of CO_3Aps is discussed in Section 4.5.1.

1.8.3 Chemical composition

The early studies of the Ca/P molar ratio of ACPs reported values close to 1.50 [332,333,342]. However, in a detailed study of the composition of ACP, it was shown [335] that ACP formed in the pH range 6.6 to 10.6 varied in Ca/P molar ratio from 1.18 to 1.50, but if the precipitates were washed before freeze-drying, the Ca/P molar ratios were 1.50±0.03 (except for the very lowest

part of the pH range). Washing also considerably reduced the percentage of the total phosphorus present as acid phosphate from 33 to 18 % at pH 6.6, and from 10 to 5 % at pH 10.6 (determined from the pyrophosphate formed on pyrolysis, Section 3.6.2). Neither the increase in Ca/P ratio nor the reduction in acid phosphate on washing could be attributed to trapped supernatant ions. It was concluded that ACPs should be recognised as a class of salts having variable chemical, but identical glass-like properties [335]. On the other hand, others have reported that the departure from a Ca/P molar ratio of 1.5 can be attributed to surface adsorbed soluble phases that can be washed away, or to occluded Ca^{2+} ions [343].

ACPs with Ca/P molar ratios of 1.43±0.01, 1.5±0.003 and 1.53±0.01 have been prepared by rapid precipitation at pH 8.9, 9.9 and 11.7 with formulae $Ca_{8.38}(HPO_4)_{1.11}(PO_4)_{4.89}(OH)_{0.27}$, $Ca_{9.00}(HPO_4)_{0.16}(PO_4)_{5.84}(OH)_{0.16}$ and $Ca_{9.21}(HPO_4)_{0.08}(PO_4)_{5.92}(OH)_{0.50}$ respectively (analyses based on pyrophosphate formation on heating, Section 3.6.2) [92,328]. About half the total phosphorus in an ACa,MgP prepared at pH 7 [340], and three quarters in a magnesium-free ACP prepared at pH 6.5 [341] were protonated. The determinations were based on an oxalate precipitation/titration method (Section 3.6.2) with the assumption that OH$^-$ ions were absent from the solid, so the acid phosphate content is a minimum value. The chemical analyses of these ACPs gave a (Ca + Mg)/P molar ratio of ~1.2 [341].

An ACP corresponding to an amorphous OCP with a Ca/P molar ratio of 1.33 has been reported [146]. This was prepared at 37 °C by rapid precipitation from an alcoholic phosphate solution (($NH_4)_2HPO_4$ 30 mmol, H_2O 250 ml, C_2H_5OH 295 ml, NH_3 solution density 0.92 g cm^{-3} 45 ml) by a calcium solution ($Ca(NO_3)_2$ 30 mmol, H_2O 100 ml, C_2H_5OH 100 ml). Following precipitation, washing with an alcoholic solution (H_2O 180 ml, NH_3 solution density 0.92 g cm^{-3} 30 ml, C_2H_5OH 210 ml), the solid was freeze dried. Chemical analyses gave Ca 31.8±0.1, P 18.5±0.1, C≤0.1 and N 0.3±0.1 wt %. These results, together with the determination of pyrophosphate on heating, gave a formula $Ca_8(PO_4)_4(HPO_4)_2$ (no H_2O given in formula).

1.8.4 Transformation reactions
Transformation in the absence of an aqueous phase

An important distinction between the transformation of ACP in the absence and presence of an aqueous phase is that in the former situation, there can be no change in the Ca/P ratio. Thus moist ACP cake (synthesis and formulae given in previous section) converts to a ns-OHAp at room temperature in about 24 h (much faster at higher temperatures) [92,328,329]; even vacuum dried samples slowly convert to apatite over a period of months at ambient temperature [344].

3.3 Preparation of stoichiometric hydroxyapatite powders

3.3.1 Introduction

The large number of different synthetic routes and published preparations demonstrates the difficulty of making s-OHAp. The variability that is found in such products has been reviewed [620].

Most synthetic methods eventually have to rely on the assumption that s-OHAp is the equilibrium phase under the preparation conditions used. Although this may be approximately true, there is no thermodynamic reason why an apatitic equilibrium phase should be exactly stoichiometric. The stoichiometry has to be determined experimentally by accurate chemical analyses and preferably also from the unit cell contents calculated from lattice parameter and density measurements (Section 3.6.3). What evidence there is, suggests that the equilibrium phase is not generally exactly stoichiometric. In studies of the phase diagram at 825 °C and 1000 bar (100 MPa), it was found that excess calcium reduced the a-axis parameter slightly, and excess water had the opposite effect [17]. At 2000 bar (200 MPa) and between 300 and 600 °C, slight changes in the a-axis parameter (range 9.415(7) to 9.4225(5) Å) have been found, depending on temperature and composition [18]. For OHAp formed at the singular point with DCPA or β-$Ca_2P_2O_7$, the a-axis parameter increased with temperature, but at the singular point with $Ca(OH)_2$, it decreased with temperature. A broad existence region at high temperatures has been found for the water content (present as OH^- ions) of OHAp, but the region was limited for changes in CaO/P_2O_5 ratio [621] (Section 3.4.3). On the other hand, the synthesis of an OHAp with excess $Ca(OH)_2$ at 1000 °C has been reported [37] (Section 3.4.2).

There do not seem to have been any precise investigations at near normal temperatures and pressures of the stoichiometry of the OHAp at equilibrium in the $Ca(OH)_2$–H_3PO_4–H_2O system, but there is no reason why the generally small deviations in stoichiometry found at higher temperatures and pressures should not also occur under milder conditions. The fact that OHAps prepared in boiling water by the prolonged hydrolysis of DCPD have a slightly enlarged a-axis parameter and are hexagonal rather than monoclinic [620] suggests that there is indeed a small variable compositional range at equilibrium, caused by a limited solubility of $Ca(OH)_2$. It seems then that, at equilibrium, the precise composition may depend significantly on temperature (and pressure). In addition to these conditions, there will be many instances when deviations (sometimes quite large) are caused by kinetic factors. As a result of these considerations, there can be no precise boundary between the preparation of s-OHAp (this section) and Ca-def OHAps in aqueous systems (Section 3.4.1).

One method that has been used to prepare s-OHAp is to use accurately stoichiometric quantities for the synthetic reaction, and attempt to ensure formation of a single phase without unwanted gain or loss of chemical species (Section 3.3.2). Another approach is to use the aqueous system $Ca(OH)_2$–H_3PO_4–H_2O at a composition in which OHAp (assumed to be stoichiometric) is the only stable phase, and to attempt to ensure that the system is at equilibrium (Section 3.3.3). The calcium and phosphorus can, for example, come from the controlled addition of suitable solutions, or from a more acidic CaP that hydrolyses to OHAp; in some systems, calcium is released from a bound form into a phosphate solution.

Yet another technique is to synthesise a ns-OHAp under nonequilibrium conditions, and to process this to give a stoichiometric composition. For example, solid state reactions have been used in which samples of nearly stoichiometric compositions are heated to a high temperature so that s-OHAp is formed as the major phase, together with a minor phase (CaO or β-TCP, depending on whether the Ca/P molar ratio is greater or less than 1.667 respectively) in an amount dependent on the departure of the overall composition from s-OHAp (Section 3.3.4). The minor phase is then extracted from the product. A further method is to rely on the easier synthesis of a related stoichiometric apatite (s-ClAp or s-BrAp) that can be converted to s-OHAp (Section 3.3.4).

3.3.2 Syntheses based on theoretical compositions

This method can be used for solid state high temperature reactions or reactions that involve precipitation. The reactants can be analysed for calcium and phosphorus, or chosen to have a well-defined composition. Analytical grade $CaCO_3$, dried at up to 500 °C, can be used without analysis, and dissolved in acid if a solution is required. Alternatively, it can be heated to constant weight at 1000 °C to provide CaO, which can be dissolved in CO_2-free water to give $Ca(OH)_2$ (great care is required to prevent absorption of CO_2). A source of phosphorus of known composition is more difficult. Analytical grade H_3PO_4 can be titrated against standard NaOH, or its composition deduced from its density [622]. DCPA or DCPD can also be used as starting materials if their compositions are known, or their purity can be established (DCPD is less favoured because of its slightly uncertain water content). For solid state reactions, it is preferable to use the better defined $Ca_2P_2O_7$, which is prepared by heating DCPA (Section 1.5.6) or DCPD (Section 1.4.7).

For quantitative reactions in solution, the reactants must be H_3PO_4 and $Ca(OH)_2$, or salts of these with ions that are unlikely to be incorporated in the apatite lattice. Thus, nitrates should be used in preference to chlorides, and ammonium salts instead of sodium or potassium salts. (It has alternatively been

claimed that K^+, NH_4^+, and NO_3^- ions are not incorporated into crystalline apatites [623], but there is evidence at least for the limited incorporation of K^+ ions [181], Section 3.4.1, and very limited incorporation of NH_4^+ ions [94,434,435,624,625], Section 4.5.4.) The pH should likewise be controlled by use of NH_4OH or NH_3 gas, rather than NaOH or KOH. An advantage of using ammonium and nitrate salts is that NH_4NO_3 can readily be removed from the separated precipitate by decomposition into gaseous products on heating. Care must be taken to exclude atmospheric CO_2 during precipitation, particularly under alkaline conditions, as CO_2 is very easily incorporated into apatites. Although this can be removed afterwards by heating in steam at 900 °C, PO_4^{3-} ions displaced by CO_3^{2-} ions from the apatite might be lost whilst filtering the precipitate.

Owing to the very low solubility of apatites, the separated precipitate will contain essentially all the original calcium and phosphorus, so it must have the correct Ca/P ratio. If precipitation has been carried out at a high pH, so that there is essentially no acid phosphate in the solid, charge balance dictates that the solid also has the correct proportion of OH^- ions. However, if precipitation is carried out near neutral pH, in principle, there could be acid phosphate present (Equation 3.14, in a direction right to left), in which case the proportion of OH^- ions will be higher than the stoichiometric value. Regardless of the pH of formation, the solid will contain a large amount of water. If the solid is now heated, there is the possibility of removing excess water, volatilisation of NH_4NO_3 if present, transformation of HPO_4^{2-} to PO_4^{3-} ions via a pyrophosphate intermediate, and removal of any small amounts of carbonate contamination. However, there is also the possibility of the formation of O^{2-} ions from the reaction

$$2OH^- \rightarrow O^{2-} + H_2O \qquad\qquad 3.1$$

with the result that the product does not have the stoichiometric OH^- ion content. Studies of the thermal decomposition of OHAp [19,21,621] (Section 3.4.3) indicate that this will occur only to a minimal extent at 900 °C in steam at atmospheric pressure (0.1 MPa). At this temperature, HPO_4^{2-} ions will be already transformed to PO_4^{3-} ions, and any carbonate should be lost, given sufficient time.

In a quantitative solution preparation of OHAp [626], 300 ml of a solution containing 0.33 mol of $Ca(NO_3)_2.4H_2O$ were brought to pH 12 with CO_2-free ammonia solution and diluted to 600 ml. 500 ml of a solution containing 0.2 mol of $(NH_4)_2HPO_4$, similarly brought to pH 12 and diluted to 800 ml, were added slowly with vigorous stirring. The reaction mixture was boiled for 10 min to improve filtration properties. The precipitate was allowed to settle, the supernatant poured off, then it was filtered. Excess water was removed by

heating to 900 °C. No lattice parameters or chemical analyses were reported. However, OHAp prepared by a similar method [627], in which gravimetric analyses of the starting materials and product were made [77], gave CaO 55.68, P_2O_5 42.32 and H_2O (by subtraction) 2.00 wt % (theoretical 55.79, 42.39 and 1.80 wt % respectively). The CO_2 content was <0.1 wt %. The lattice parameters were $a = 9.422\pm0.003$ and $c = 6.883\pm0.003$ Å [4I76].

In solid state quantitative reactions, the main problem is to ensure complete reaction to a single phase. It is of little importance what starting materials are used, except insofar as this affects the accuracy of the composition determination. Starting materials that have been used include $CaCO_3$, CaO, $Ca(OH)_2$, DCPD, DCPA and $Ca_4P_2O_9$. To minimise formation of O^{2-} ions, the final high temperature heating should be carried out in steam.

The following scheme has been used to prepare s-OHAp by quantitative solid state reactions [628]. DCPA was heated at 1000 °C in air for 12 h to give $Ca_2P_2O_7$ (Section 1.4.7). Weighed amounts of $Ca_2P_2O_7$ and $CaCO_3$ in the mole ratio 3:4 were ground in acetone in a steel ball-mill to a 10 μm particle size, pelleted to ensure completion of subsequent reactions, and dried at 200 °C. These were then heated under vacuum at 1100 °C for 20 h in a platinum crucible to remove CO_2:

$$3Ca_2P_2O_7 + 4CaCO_3 \rightarrow 10CaO.3P_2O_5 + 4CO_2: \qquad 3.2$$
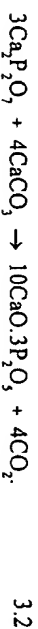
The weight loss was 15.11±0.00 wt % (theoretical 15.14). The pellets were then heated at 900 °C for 24 h in a platinum crucible in a stream of steam at 1.5 atm (0.15 MPa). The water weight gain was 1.801±0.003 wt % (theoretical 1.826) which indicated an OH^- ion content of 98.6±0.2 % of the theoretical.

The phosphorus content of the OHAp was 18.50 wt % (theoretical 18.49). A dissolution and titration method of analysis gave the OH^- ion content to within ±5% of theoretical [623] (Section 3.6.1). XRD showed a single phase of well-crystallised monoclinic OHAp with pseudohexagonal lattice parameters $a = 9.4182(5)$ and $c = 6.8814(4)$ Å (BO Fowler, personal communication, 1991). Two samples prepared by a similar, but not identical method, were also monoclinic with $a = 9.4170(3)$ and $c = 6.8796(3)$ Å for a single phase sample, and $a = 9.4175(2)$ and $c = 6.8802(2)$ Å for a sample that contained some $Ca(OH)_2$ [620]. Quantitative IR of the OH^- stretching band indicated a 17 % OH^- ion deficiency in the first sample, and 1 % in the second [620]. The three pseudohexagonal lattice parameter determinations agree well, and their means ($a = 9.4176$, $c = 6.8804$ Å, estimated error in both ±0.0005 Å) probably give the best estimate of the lattice parameters for monoclinic OHAp.

3.3.3 Equilibrium syntheses in solution

This method assumes that s-OHAp is the equilibrium phase under the

conditions used (see Section 3.3.1) and that the system is in equilibrium. Two approaches can be used. In the first, the composition must be controlled so that the solution is supersaturated only with respect to OHAp, and not to any other CaP. This condition requires the use of constant composition methods (Section 3.8) which can generally only be used for small quantities.

The second approach is to assume that s-OHAp is the most stable phase in solution (Fig. 1.1) at a pH above the OHAp/DCPD singular point (pH 4.3 at 25 °C, Section 1.4.6). If this assumption is correct, any CaP left in an aqueous solution for long enough should transform to s-OHAp, provided the pH is not allowed to fall below the singular point. In practice, it is usual to hydrolyse DCPD or DCPA, rather than a Ca-def OHAp, boil to speed the reaction, and to periodically replace the water to remove H+ ions. This type of preparation seems often to lead to a ns-OHAp or Ca-def OHAp with a slightly expanded a-axis parameter [620], and for this reason is discussed in the section on these compounds (Section 3.4.1).

An alternative to hydrolysis is to directly precipitate OHAp above the OHAp/DCPD singular point. This reaction is again usually done in boiling water to promote equilibrium. An early example was the precipitation of OHAp by the addition of a saturated solution of $Ca(OH)_2$ to a dilute H_3PO_4 solution (14 g l^{-1}) until alkaline to phenolphthalein (pH >9) [75]. To avoid DCPA precipitation, neutralisation was initially carried out in the cold, the mixture boiled and further $Ca(OH)_2$ added until the red colour change was stable for 1 h. The precipitate (separated by filtration and dried at 80 °C) had a CaO/P_2O_5 mol ratio of 3.5 to 3.6 (theoretical 3.33) and an XRD pattern of OHAp after heating at 800 °C.

In most of the subsequent preparations of OHAp by reaction between $Ca(OH)_2$ and H_3PO_4, acid has been added to alkali to avoid formation of metastable phases, and a slurry instead of a solution of $Ca(OH)_2$ used to increase the yield. Of two such almost identical preparations [38,629], the more recent will be described in detail. A boiling aqueous suspension of $Ca(OH)_2$ (194 g of CaO, made by calcining $CaCO_3$ at 1000 °C for 24 h, then added to 7 l of freshly boiled distilled water) was titrated with H_3PO_4 (0.5 mol l^{-1} recrystallised $2H_3PO_4.H_2O$, standardised by titration) [38]. These were reacted in a 15 l vessel equipped with a reflux condenser, stirrer and ports for introduction of N_2 and acid, which was added at 1 ml per min until a Ca/P molar ratio of 1.70 was reached. After this, boiling and stirring was continued for a further two days, the solid allowed to settle, and the supernatant siphoned off. 5 l of freshly boiled distilled water were then added, and the mixture boiled and stirred for two days to remove excess $Ca(OH)_2$. The supernatant was then syphoned off, and the process repeated four times, the last using dilute H_3PO_4 (0.001 mol l^{-1}). The product was then dried at 110 °C in a stream of dry N_2. The mean refractive index was 1.636, and IR indicated that it was

essentially free of carbonate. Chemical analyses gave Ca 39.25 and P 18.13 wt % (theoretical 39.89 and 18.50 wt % respectively), hence the Ca/P molar ratio was 1.67 (standard error 0.042). The BET surface area was 16.7 m^2 g^{-1} [38]. Lattice parameters of $a = 9.4174(2)$ $c = 6.8853(2)$ Å, 90% of theoretical OH content determined by IR, and a full c-axis channel from Rietveld structure analysis have been reported for this preparation [620]. These lattice parameters agree well with those given in the previous section. The solubility product is given by Equation 3.23 in Section 3.9.

OHAp has been prepared [630] by adding equal volumes of CO_2-free $Ca(OH)_2$ and MCPA solutions at a constant rate to rapidly stirred distilled-deionised water at 100 °C with CO_2-free N_2 flushed through the system. The precipitate was then filtered under CO_2-free N_2, washed with acetone or distilled-deionised water, and dried at 105 °C. Well-defined prism-shaped crystals from 0.7 to 7 μm long and 0.04 to 0.14 μm wide [631] were formed, depending on the rate of addition of the solutions [630]. Products with surface areas of 9.1 and 26.6 m^2 g^{-1} contained Ca 39.4 and P 18.2 wt % and Ca 38.8 and P 18.1 wt % respectively (theoretical Ca 39.89 and P 18.50 wt %) [630]. A preparation with a surface area of 20.0±0.4 (standard error, SE) m^2 g^{-1} had lattice parameters $a = 9.422±0.001$ (SE) and $c = 6.883±0.003$ (SE) [632] and $a = 9.422±0.002$ (standard deviation, σ) and $c = 6.887±0.002$ (σ) [631]. A similar process to the above has been described for large scale production of OHAp [633].

OHAp can also be prepared by methods similar to those given above in which precipitation takes place in the presence of ions additional to those in the $Ca(OH)_2$-H_3PO_4-H_2O system [634,635]. 2.5 l of $(NH_4)_2HPO_4$ solution (7.25 × 10^{-2} mol l^{-1}) and 2.5 l of $Ca(NO_3)_2.4H_2O$ (13.33 × 10^{-2} mol l^{-1}) both at pH 8.5 to 9 (adjusted with NH_3 gas) were pumped at 100 ml h^{-1} into a 22 l flask containing 9 l of CH_3COONH_4 (1 mol l^{-1}) [634]. The reaction mixture was vigorously boiled during addition of the solutions and for 3 h thereafter, and the pH was maintained at 8.5 to 9 (measured at room temperature) with NH_3. After the solid had settled, the supernatant was removed and replaced by 9 l of boiled distilled water and the mixture boiled for 2 h. This process was repeated a few times to remove soluble salts. Extensive precautions were taken against ingress of CO_2. The precipitate was filtered and dried at 150 °C for 48 h, then at 1000 °C in air. Chemical analyses were: P 18.22±0.27 and Ca 39.63±0.99 wt % (theoretical 18.50 and 39.89 wt % respectively) and 0.04 wt % CO_2. OHAps with specific surface areas from 6.39 to 50.1 m^2 g^{-1} have been prepared by a similar precipitation process by varying the conditions of temperature and seeding, and the concentration and rates of addition of the reagents [635]. This method has also been used to prepare OH,FAps [636] (Section 3.4.4).

Preparation of OHAp at 70 °C by the addition of 2.9 l of a potassium phosphate buffer (0.0805 mol l⁻¹ K_2HPO_4, 0.0195 mol l⁻¹ KH_2PO_4, pH 7.4) over 30 min to 11.6 l of a solution containing 0.477 mol of $Ca(NO_3)_2$ and 0.318 mol of KOH, followed by stirring for one month has been described [637]. The product was analysed in several different laboratories by a number of methods including XRD, IR (Section 3.6.1), BET, chemical analysis, differential thermal analysis, NMR (Sections 3.6.1, 3.6.2 and 3.12) and transmission electron microscopy. The Ca/P molar ratio was in the range 1.631 to 1.659 and the lattice parameters were $a = 9.428$ and $c = 6.882$ Å. There was a deficiency in the OH⁻ ion content of about 10 % (Section 3.6.1). About 2 % of the total phosphate was protonated and thought to be on the surface.

3.3.4 Miscellaneous methods

OHAp has been prepared by heating a stoichiometric mixture of minus 200-mesh MCPM and $CaCO_3$ for 3 h at 1200 °C in an atmosphere of equal volumes water and N_2 [16]. If the system is at equilibrium and the overall composition deviates from that of s-OHAp (assumed to be the equilibrium phase), a small amount of a second phase will be formed ($Ca(OH)_2$ for excess calcium, and TCP for excess phosphorus). The reaction mixture was then extracted twice with neutral ammonium citrate solution [638] to remove nonapatitic CaPs (this would also remove any $Ca(OH)_2$), washed thoroughly, and dried at 110 °C. Neither lattice parameters nor the chemical composition were reported.

s-OHAp can in principle be precipitated in solution under conditions that are some way from equilibrium, provided these have been chosen correctly. These conditions have been studied systematically for OHAp precipitated by slow addition, during T min, of 400 ml of $(NH_4)_2HPO_4$ solution (0.25 mol l⁻¹) containing V_1 ml of NH_4OH solution (density 0.92 g ml⁻¹) to 900 ml of $Ca(NO_3)_2$ solution (0.20 mol l⁻¹) containing V_2 ml of NH_4OH (density 0.92 g ml⁻¹) in a 3 l flask fitted with a Liebig condenser [537]. During time T, and for a further 15 min, the reaction mixture was boiled and stirred, after which the precipitate was separated by filtration and dried overnight at 105 °C. Although stoichiometry could have been explored as a function of many experimental variables, only three were chosen (Equation 3.3). It was found that the Ca/P molar ratio was equal to $1.688 + 0.020x_1 + 0.024x_2 - 0.019x_1x_2$ to a good approximation for a fixed time T of 180 min. The coefficients, x_1 and x_2, were determined by a least squares procedure. Conditions for the synthesis of s-OHAp yielded a product, after heating at 900 °C for 5 h, with a Ca/P molar ratio of 1.67±0.01 and lattice parameters $a = 9.420±0.002$ and $c = 6.880±0.002$ Å.

OHAp has been prepared by boiling $CaSO_4$ with strongly alkaline Na_3PO_4 ...

$$x_1 = \frac{(V_1 - 80)}{40}$$

$$x_2 = \frac{(V_2 - 100)}{60}$$

$$\text{and } x_3 = \frac{(T - 180)}{60}$$

3.3

[75,620] or BrAp [80,576] in steam at about 800 to 1000 °C. The product from ClAp is monoclinic [400,620] (Fig. 3.1); OHAp from BrAp has not been investigated, but is likely to be also monoclinic. As both ClAp (Sections 2.4.1 and 2.5.2) and BrAp [50] can be easily produced in a well-crystallised form, this approach, although little used, should be a good method for producing substantial quantities of monoclinic OHAp with micrometre or larger crystals (0.4 mm twinned monoclinic crystals of OHAp have been made from ClAp [613], Section 3.5).

Oriented crystals of OHAp can be produced by the hydrolysis of oriented preparations of OCP grown on a cation-selective membrane [164] (Section 1.3.5).

A variety of different methods have been developed for the production, sometimes on a large scale, of OHAp for use directly, or after further processing, as a bone replacement material or for the coating of metal prostheses [105,597,633,640-642] (and references therein). These biomaterials applications are discussed further in Section 4.6.7.

3.4 Preparation of other apatites with hydroxyl ions
3.4.1 Calcium-deficient and nonstoichiometric apatites

There is no difficulty in preparing Ca-def and ns-OHAps in aqueous systems, the problem lies in obtaining a single phase product with larger than 1 μm crystals, so that the bulk composition will not be dominated by that of the surface. As discussed in Section 3.3.1, there seems to be a small composition range possible for OHAp, even under equilibrium conditions, so that some of the methods described previously for s-OHAp and some hydrothermal methods [17,18] can be used to produce slightly ns-OHAps.

A Ca-def OHAp has been prepared by the simultaneous addition of $Ca(CH_3COO)_2$ and $(NH_4)_2HPO_4$ solutions (both 0.1 mol l⁻¹) at a rate equivalent to 2.5 mmol of $Ca_3(PO_4)_2$ per hour to boiling CH_3COONH_4 at pH 4.5 with constant stirring [643]. The mixture was refluxed for 16 to 20 h before filtration, and the precipitate washed with cold water and C_2H_5OH before drying. The initial precipitate was OCP, but the final product only contained ...

a thickness much less than the width. The Ca/P molar ratio was about 1.56 and the lattice parameters a little different (a +0.16% and c -0.09%) from those of sintered OHAp. If these are taken as a = 9.418 and c = 6.881 Å (Table 1.4), the ns-OHAp lattice parameters were a = 9.433 and c = 6.875 Å. The diffraction pattern was unchanged after heating at 330 °C, above 450 °C, a small amount of α-Ca₃P₂O₇ was seen, and after heating at 600 °C, the lattice parameters changed to those of sintered OHAp. For a Ca/P molar ratio extrapolated to 1.5, the weight loss on heating indicated a water content of 2.5 to 3.5 mol per unit cell. There was a 25 % phosphorus as pyrophosphate yield on heating at 600 to 650 °C, again for a Ca/P ratio extrapolated to 1.5. The lattice contraction on heating was attributed to loss of lattice water (Section 3.7.3).

Ca-def OHAps have been prepared in the pH range 6.4 to 13.38 by slow release of Ca²⁺ ions by boiling off water from a phosphate solution containing Ca²⁺ ions chelated to ethylenediaminetetra-acetic acid (EDTA) [181]. 60 g of EDTA and 60 g of DCPA or Ca₃(PO₄)₂ were mixed in 1.8 l water and brought to the desired pH with alkali, usually KOH. After stirring for 6 h to allow maximal dissolution, excess solids were removed by filtration. The volume was reduced to one-sixth by boiling whilst N₂ was bubbled through, then the crystals were separated, washed, and dried at 110 °C for 12 h. The preparations all had an enlarged a-axis parameter compared to normal OHAp (up to a = 9.45 Å), and the c-axis parameter seemed to be related to the pH of formation. For pH < 7, c was slightly smaller, and in alkaline conditions, slightly larger than in normal OHAp. One sample prepared at pH 6.96 had a composition (in wt %) of: K₂O 0.68, CaO 51.47, P₂O₅ 43.21, CO₂ 0.04, H₂O 4.6 (by difference), which gave Ca/P and (Ca+K)/P molar ratios of 1.51 and 1.53 respectively. Loss of water on heating and resultant lattice parameter changes are discussed in Section 3.7.3.

Hydrolysis of DCPD in boiling distilled and deionised water for one month with four to six changes to reduce acid build up, produced an apatite with a Ca/P molar ratio of 1.60 (chemically determined) and a lower Ca/P ratio than s-OHAp (by Rietveld analysis) [620]. Lattice parameters were a = 9.4290(3) and c = 6.8805(3) Å.

Ca-def OHAp with crystals of micron dimensions has been synthesised by slow hydrolysis of DCPD (80 °C, 94 h, 0.1 × 2 μm² crystals) or DCPA (90 °C, 71 h, 0.25 × 10 μm² crystals) in which the acid produced was neutralised by NH₃ from the slow hydrolysis of urea [644]. 5 l of water, containing 50 g of the solid and 9 g of urea, were adjusted to an initial pH of 4.0 with HNO₃. The pH after the reaction was 6.6 to 6.8. Chemical analysis gave a Ca/P molar ratio of 1.53±0.03, XRD showed only the presence of apatite, and after heating at 1000 °C, only β-TCP. Lattice parameters and refractive indices were not given, ... IR showed the presence of acid phosphate (band at 875, shoulders at 1180

and 1200 cm⁻¹), and a trace of carbonate. NMR studies were consistent with the chemical formula Ca₉HPO₄(PO₄)₅OH [193] (Section 3.12).

The constant composition method (Section 3.8) has been used at 37 °C to prepare nonstoichiometric apatites with Ca/P molar ratios from 1.49 to 1.65 at pH 6.0 to 9.0 [645,646]. Growth was on OHAp seeds in solutions with a Ca/P molar ratio of 1.667 and ionic strength of 0.100 mol l⁻¹ at equilibrium with respect to OCP, but supersaturated with respect to OHAp (increasingly so at higher pH). There was little difference between the IR spectrum (including the HPO₄ band at 875 cm⁻¹) and lattice parameters (a = 9.42; c = 6.88 Å) of the product compared with the seed material.

3.4.2 Calcium-rich apatites

Precipitated apatites strongly adsorb Ca(OH)₂ from lime solutions [647]. The possibility of precipitated BCaPs with a Ca/P molar ratio greater than 1.667 has been reviewed [6,648]. The method discussed earlier [537] (Section 3.3.4), in which the stoichiometry was explored as a function of precipitation conditions, gave products with Ca/P molar ratios from 1.63 to 1.73. The precipitates with a Ca/P molar ratio above 1.67 were thought to be mixtures of OHAp and Ca(OH)₂, or an apatite adsorbing Ca²⁺ ions and an equivalent amount of negative ions.

A series of calcium-rich apatites with Ca/P molar ratios above 1.67 has been prepared by heating s-OHAp and CaCO₃ in air presaturated with water vapour at 1000 °C for 10 days [537]. XRD showed only an apatitic phase whose c-axis parameter remained constant at 6.884 Å, whilst the a-axis parameter decreased linearly with excess Ca(OH)₂ from a = 9.420 to 9.373 Å at which point the Ca/P molar ratio was 1.75. Chemical tests and IR showed the absence of a separate Ca(OH)₂ phase, but above a ratio of 1.75, IR indicated its presence. The thermal stability of this new apatite under various conditions was reported [537], as well as its IR spectrum (Section 3.11.6), which showed additional OH bands. The possibility has been raised that the change in lattice parameters and the extra OH IR bands (see Section 3.11.5 for a discussion of these) might be due to accumulation of F⁻ ions from the muffle furnace (BO Fowler, personal communication, 1991).

3.4.3 Apatites with oxygen in different oxidation states

Oxyapatites (OAps) with O(I-II) as O²⁻ ions

The existence of oxyapatite, Ca₁₀(PO₄)₆O, was the subject of controversy for many years [85,86]. However, its formation now appears to be well-established, though it possibly always contains a small amount of OH⁻ ions. Perhaps for this reason, it does not occur in published high temperature phase diagrams of the CaO-P₂O₅ system (Section 1.6.6). The name "voelckerite" was coined in 1912